Analysis of Poisonous Glycols from Blood and Aqueous Samples

1 Introduction

Ethylene glycol (EG) and diethylene glycol (DEG) are two toxic glycols used in coolants and antifreezes. Triethylene glycol (TEG) is less toxic than EG and DEG, and can be used in plastics or air disinfectants. Propylene glycol (PG) or 1,2-propanediol is generally recognized as safe for use in foods, cosmetics and medicines. It can cause skin irritation and may be toxic in high doses in children.

2 Scope

This procedure allows for the screening and confirmation of blood samples for EG, DEG, TEG and PG. It also allows for the analysis of aqueous samples for EG.

3 Principle

For screening or confirmation of EG in blood samples, specimens are crashed out with acetonitrile, taken to dryness, and derivatized with heptafluorobutyric anhydride (HFBA) before analysis by gas chromatography with mass spectrometry (electron impact) [GC/MS(EI)]. For screening of multiple glycols in blood samples, or for a second test for confirming EG, samples are extracted in acetonitrile and converted to their trimethylsilyl derivatives for improved retention on a typical capillary column. Analysis of derivatized extracts is by GC/MS(EI) or GC/MS (chemical ionization) [GC/MS(CI)].

Aqueous samples are screened for EG via direct analysis in real time (DART) time of flight mass spectrometry (TOFMS). Positive findings will be confirmed via GC/MS.

4 Specimens

This procedure is validated for multiple glycols in blood. It is also validated for EG in aqueous samples.

5 Equipment/Materials/Reagents

a. Gas Chromatograph / Mass Spectrometer (GC/MS) capable of EI and CI ionization and equipped with a 30 m x 0.25 mm x 0.25 µm film thickness DB-5 (or equivalent) column (dedicated to silyl derivatives)

b. Gas Chromatograph / Mass Spectrometer (GC/MS) equipped with a 30 m x 0.25 mm x 0.25 µm film thickness DB-5 (or equivalent) column

c. Vortex mixer

d. Centrifuge

e. Evaporator with nitrogen

f. Heating block

g. Adjustable volume pipettes (0.025 mL to 1 mL) with appropriate tips

h. Routine laboratory supplies, including 12 x 75 mm test tubes, autosampler vials with crimp caps, disposable glass pipettes, test tube racks, graduated cylinders, parafilm etc.

i. Acetonitrile (HPLC grade)

j. Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA/TMCS) (obtained from Sigma-Aldrich Chemical Company, or an equivalent supplier)

k. Ethyl acetate (HPLC grade)

l. Direct Analysis in Real Time Time-of-Flight Mass Spectrometer (DART TOFMS)[1]

m. Heptafluorobutyric anhydride (HFBA), ≥99%, for GC derivatization

n. Hexane (UV grade)

o. Sodium sulfate (Reagent grade)

6 Standards and Controls

a. Ethylene glycol (EG) Stock Standard (10 mg/mL):
Ethylene glycol traceable to United States Pharmacopoeia (USP) can be purchased from USP or another approved vendor. Storage and stability are determined by the manufacturer. Add 100 mg of EG to a 10-mL volumetric flask. Bring to the mark with acetonitrile and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

b. EG Working Stock (500 µg/mL):
Add 0.5 mL of the EG Stock Standard (10 mg/mL) to a 10-mL volumetric flask. Bring to the mark with acetonitrile and mix well to dissolve. Store refrigerated in glass; stable for at

[1] As of the time of the issuance of this procedure, only DART-1 has been validated for this application.

least 1 year.

c. Diethylene glycol (DEG) Stock Standard (1 mg/mL):
Diethylene glycol can be purchased from Sigma-Aldrich or another approved vendor. Storage and stability are determined by the manufacturer. Add 100 mg of DEG to a 100-mL volumetric flask. Bring to the mark with acetonitrile and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

d. Triethylene glycol (TEG) Stock Standard (1 mg/mL):
Triethylene glycol can be purchased from Sigma-Aldrich or another approved vendor. Storage and stability are determined by the manufacturer. Add 100 mg of TEG to a 100-mL volumetric flask. Bring to the mark with acetonitrile and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

e. Propylene glycol (PG) or 1-2 Propanediol Stock Standard (1 mg/mL):
Propylene glycol can be purchased from Sigma-Aldrich or another approved vendor. Storage and stability are determined by the manufacturer. Add 100 mg of PG to a 100-mL volumetric flask. Bring to the mark with acetonitrile and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

f. Negative Control Blood:
Purchased from Cliniqa or another approved vendor. Storage and stability are determined by the manufacturer. A Negative Control Blood sample is analyzed with every blood assay.

g. Positive Control Blood for BSTFA Derivatization (100 µg/mL):
Positive Controls will be prepared fresh. When 25 µL of the appropriate 1 mg/mL stock solution, or 50 µL of the EG Working Stock (500 µg/mL) is added to 0.25 mL Negative Control Blood, the resulting control is 100 µg/mL. A Positive Control will be prepared for each analyte of interest. TEG and DEG are routinely combined into one Positive Control, while EG and PG are typically analyzed individually.

h. d_4-Ethylene glycol (d_4-EG) Internal Standard Stock Standard (2.5 mg/mL):
d_4-Ethylene glycol can be purchased from Isotec or another approved vendor. Storage and stability are determined by the manufacturer. Add 25 mg of d_4-EG to a 10-mL volumetric flask. Bring to the mark with acetonitrile and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

i. d_4-Ethylene glycol (d_4-EG) Internal Standard Solution (500 µg/mL):
Add 2.0 mL of d_4-EG Internal Standard Stock Standard (2.5 mg/mL) to a 10-mL volumetric flask. Bring to the mark with deionized water and mix well to dissolve. Store refrigerated in glass; stable for at least 1 year.

j. Positive Control Blood for HFBA Derivatization (EG at 72 µg/mL and 1200 µg/mL): Positive Control Blood will be prepared on the day of analysis as described in Table 1.

Table 1: Blood Control Preparation

Ctl Level (µg/mL)	Blood Volume (mL)	µL EG Working Stock (500 µg/mL)
72	0.25	36
1200	0.25	600

k. Negative Control Water:
Obtained from an appropriate commercial source or from the in-house tap. A Negative Control Water sample is analyzed with every water assay.

l. Positive Control Water (EG at 100 µg/mL):
Add 0.01 mL of the EG Control Stock Standard (10 mg/mL) to 0.99 mL of Negative Control Water. Prepare fresh. When sample size permits, an unknown sample can also be spiked with the EG Stock Standard as an additional Positive Control sample. A Positive Control Water sample is analyzed with every water assay.

7 Sampling

Not applicable.

8 Procedure

8.1 Screening for EG in Aqueous Matrices

a. Control and unknown samples are analyzed directly in duplicate on the DART-TOF MS using the instrumental parameters in Section 9.1 of this procedure. (No sample preparation is necessary.)

8.2 Screening or Confirmation of EG in Blood Specimens (HFBA Derivative)

Appendix 1 contains an abbreviated version of this part of the procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. Label centrifuge tubes for each sample and control.

b. Aliquot 0.25 mL of negative control blood.

c. Aliquot 0.25 mL of each case sample(s) in duplicate.

d. Prepare the positive controls in duplicate as directed in 6.j.

e. Add 50 µL of the d_4-EG Internal Standard Solution (500 µg/mL) to one replicate of each sample and control. Note: The replicate without internal standard will be used for ion ratio comparison.

f. Bring the total volume in the centrifuge tube to approximately 1.5 mL with acetonitrile.

g. Vortex well. Centrifuge at approximately 10,000 rpm for 5 minutes.

h. Remove the acetonitrile layer to a labeled 12 x 75 test tube. Evaporate to dryness under nitrogen at 50°C.

i. Reconstitute extracts in 0.1 mL acetonitrile and vortex well.

j. Add 50 µL HFBA. Cap with a snap cap and parafilm and vortex well.

k. Heat at 60°C for 30 minutes. Cool to room temperature.

l. Vortex with 0.5 mL hexane and 0.5 mL deionized water.

m. Centrifuge for 1 minute at approximately 3000 rpm. Remove hexane layer to a labeled 12 x 75 test tube. Add a small scoop of sodium sulfate (approximately 0.2 g) and vortex.

n. Remove 0.05 mL of the hexane layer to a labeled autosampler vial. Add 0.1 mL hexane to each autosampler vial.

o. Analyze 1 µL by GC/MS(EI) using the parameters in Section 9.2 after ensuring that hexane is in the autosampler rinse vials.

8.3 Confirmation for EG, DEG, TEG and PG in Blood and Confirmation of EG in Aqueous Samples

a. Add 0.25 mL of specimen or control to an appropriately labeled 12 x 75 mm test tube.

b. Spike positive controls, as appropriate.

c. Add 0.5 mL acetonitrile to each sample.

d. Cap and vortex for approximately 20 seconds.

e. Centrifuge at approximately 2500 rpm for 2 minutes.

f. Remove supernatant to a new 12 x 75 mm test tube.

g. Evaporate the organic layer to dryness with nitrogen at approximately 40°C.

h. Reconstitute the residue with 50 µL BSTFA/TMCS.

i. Cap tubes and incubate all samples at approximately 60°C i a heating block for at least 30 minutes.

j. Allow extracts to cool down to room temperature. Transfer extracts to autosampler vials. Analyze 1 µL by GC/MS(EI) or (CI) using the instrumental parameters in Section 9.3 of this procedure. It is important to analyze the extracts on a GC column that is dedicated to silyl derivatives. To compensate for known carryover withi this procedure, ethyl acetate blanks and BSTFA/TMCS blanks should precede every unknow sample.

9 Instrumental Conditions

9.1 DART-TOF MS Analysis

9.1.1 DART Ionization Source Parameters:

Anode Polarity:	Positive (+)
Needle Voltage:	3999 V
Electrode #1 Voltage:	75 V
Electrode #2 Voltage:	150 V
Gas Control:	~ 2.4 LPM
Temperature Control:	set 410°C (actual ~ 400°C)

9.1.2 TOF-MS Parameters:

Tune File: DART +	
Needle Voltage:	V
Ring Lens Voltage:	5 V
rifice 1 Voltage:	30 V
rifice 2 Voltage:	5 V
Peaks Voltage:	300 V
Mass Range:	43-500 *m/z*

9.2 GC/MS Parameters for HFBA Derivative

Appendix 2 contains an abbreviated version of the instrumental conditions in Section 9.2 and 9.3 of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

9.2.1 GC Paramaters

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temperature 1	40°C	inlet temperature	300°C	type	DB-5
hold 1	1 min	injection mode	split	length	30 m
ramp 1	10°C/min	split	10:1	internal diameter	0.25 mm
temperature 2	130°C	carrier gas	ultrapure helium	film thickness	0.25 µm
ramp 2	30°C/min	carrier mode	constant flow		
temperature 3	325°C	flow	1.2 mL/min		
hold 2	1.5 min				

9.2.2 Mass Spectrometer Parameters

ionization mode	electron impact (+)	source temperature	230°C
scan mode	full scan	transfer line temperature	280°C
scan range	35 - 500 m/z	quad temperature	150°C
		solvent delay	5.0 min

9.3 GC/MS Parameters for BSTFA Derivatives

9.3.1 GC Parameters

Oven Parameters		Inlet and Carrier Parameters		Column Parameters	
temperature 1	60°C	inlet temperature	250°C	type	DB-5
hold 1	2 min	injection mode	splitless	length	30 m
ramp 1	10°C/min	carrier gas	ultrapure helium	internal diameter	0.25 mm
temperature 2	180°C	carrier mode	constant flow	film thickness	0.25 µm
ramp 2	35°C/min				
temperature 3	250°C				
hold 2	10 min				

9.3.2 Mass Spectrometer Parameters (EI Analysis)

ionization mode	electron impact (+)	source temperature	230°C
scan mode	full scan	transfer line temperature	270°C
scan range	70 - 500 m/z	solvent delay	5.0 min
		quad temperature	150°C

9.3.3 Mass Spectrometer Parameters (CI Analysis)

ionization mode	methane chemical ionization (+)	source temperature	230°C
scan mode	full scan	transfer line temperature	270°C
scan range	70 - 500 m/z	solvent delay	5.2 min
		quad temperature	150°C

10 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this procedure. In general, compound identification will be based on comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard or Positive Control. In most cases, all of the below should be met in order to identify one of the target analytes within a biological specimen.

10.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. It is noted, however, that derivatized glycols often produce wide chromatographic peaks on the analytical column used in this procedure. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.1.1 Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute) obtained from injection of an extracted Positive Control.

10.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10-fold

greater than that for any observed peak at a similar retention time in a Negative Control or solvent blank injected just prior to that sample.

10.2 Mass Spectrometry of HFBA derivative of EG

The following ions may be traced for ion ratio comparison of an unknown to a positive control: 169, 197, 213, 241. Only the 241 and 213 ions are from the EG; the other ions are from the HFBA. Therefore, the 169 and 197 will be present in the EI spectrum of the d_4-EG-HFBA derivative as well. For this reason, it is best to use samples with no internal standard added for ion ratio comparisons.

Detectable 255 ion in a peak eluting near the internal standard may indicate the presence of PG. If the 255 ion is detected in an unknown near the retention time of the internal standard (within a few scans), this sample should be analyzed by a different method to verify that PG is not present, as it will interfere with the quantitation of EG.

10.3 Mass Spectrometry of BSTFA derivatives (EI data)

The following ions may be traced for each analyte:

- EG: 191, 133, 103, 147
- PG: 133, 147, 117
- DEG: 103, 147, 117
- TEG: 103, 147, 161

The mass spectrum of the analyte of interest should match that of an extracted Positive Control within a reasonable degree of scientific certainty. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure (Tox 104) for further guidance.

10.4 DART-TOF MS Data

The following two ions are used to screen for EG in aqueous samples: 63.0446 and 45.0340 (EG – water). Unknown samples should be spiked with EG at a concentration of 100 µg/mL to rule out the possibility of false negative results if the sample may not be pure water and if sample size permits.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. There are only two ions in the HFBA derivative of EG that are unique to EG. The other ions that are found in the MS of the EG-HFBA derivative are HFBA ions.

b. Limit of Detection:

The limit of detection has been administratively set to 100 µg/mL for DEG, TEG and PG in blood samples.

The limit of detection has been administratively set to 25 µg/mL for EG in blood samples.

The limit of detection for EG in aqueous samples is 100 µg/mL.

c. Interferences: EG cannot be accurately identified using the HFBA derivative method in the presence of PG. Grossly decomposed or putrefied samples may affect detection limits.

14 Safety

The derivatizing reagents used in this procedure have noxious odors. They should be used in the fume hood to prevent excess exposure to their odor.

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

Guidelines for Comparison of Mass Spectra (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

FBI Laboratory Safety Manual.

Gembus, V..; Goulle, J.P.; Lacroix, C.; *J Anal Tox.* 2002**,** *26*, 280-285.

Pan, Y.M., et al.; *J Anal Tox.* 2001, *25*, 328-332.

Wurita, A., et al.; *Forensic Toxicol.* 2013, *31*, 272-280.

Rev. #	Issue Date	History
3	08/23/12	Specified use of DART-1 in Section 5k. Updated chromatography decision criteria in Section 11.1.
4	03/21/14	A new method to quantitate ethylene glycol in blood specimens was added, leading to updates in Sections 3, 5b, 5h, 5m, 5n, 5o, 6a-6d, 6i, 6k-l, 6n, 7, 9.2, 10.2, 11.2, 12, 14 and 16. In Section 6a-6g and 6j-6k, specified that standard solutions should be stored in glass and updated provider of Negative Control Blood in section 6h. Updated wording for measurement uncertainty in Section 13. Added Appendix 1.
5	06/03/16	Removed all quantitative aspects of the procedure resulting in changes to the following Sections: 2, 3, 6, 8.2, 8.3, 11, 12 and 13 as well as Appendices 1 and 2. Removed Calibration Section (Section 7) and renumbered subsequent sections. Retitled Section 12 (Measurement Uncertainty).

Approval

Redacted - Signatures on File

Appendix 1: Abbreviated version of the EG Screening and Confirmation procedure for bench use.

Redacted - Form on File

Appendix 2: Abbreviated Version of the EG Screening and Confirmation Instrumental Parameters for bench use.

Redacted - Form on File